Performance Verification of a Serological Assay for the Detection of IgG anti-Pertussis Toxin in a Public Health Laboratory

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ABSTRACT

Background: Pertussis ("whooping cough"), caused by a gram-negative coccobacillus, *Bordetella pertussis*, is a vaccine-preventable disease. The CDC listed the accumulative national figure of 29,834 cases as of September 22, 2012, compared to 15,216 of total cases in 2011 [1]. Similarly, CDC reported that the State of Maryland observed 235 cases in 2012 reported as of September 22, 2012 compared to 71 cases in January-September 2011 [1]. Laboratory confirmation of pertussis cases with clinical symptoms (onset of disease of 0-4 weeks) included culture and PCR test. The CDC in collaboration with the FDA developed the IgG Anti-Pertussis Toxin ELISA to be utilized in adults and adolescents (aged ≥ 11), who have not been vaccinated in the past 6 months as well as presenting with at least 2 weeks post-cough onset [2,3].

Objective: To conduct the performance verification of the CDC IgG Anti-Pertussis ELISA for use a state public health laboratory setting.

Method: The CDC IgG Anti-pertussis Toxin ELISA was performed following the CDC protocol [2,3].

Results: The performance verification parameters verification (accuracy, precision, reproducibility) were all met within the CDC acceptable values.

Conclusion: Implementation of this assay in a state public health laboratory setting can be a useful tool for surveillance during a pertussis outbreak.

INTRODUCTION

The pertussis toxin (PT) is a toxin consisting of A(S1) subunit and B(S2-S5) oligomer, which bind to receptors on target cells then respond by releasing antibodies [4]. Antibodies against PT develop after natural infection by pertussis or childhood vaccination. *B. pertussis* continues to frequently occur irrespective of effective vaccination [4]. Due to the current increase in reported cases, the Maryland Department of Health and Mental Hygiene Laboratories Administration Division of Virology and Immunology Vaccine Preventable Diseases Section planned to verify the performance of the CDC IgG Anti-pertussis toxin enzyme-linked immunosorbent assay (ELISA). This test detects pertussis-specific antibody titers using purified antigens specific for the PT of *B. pertussis*. Furthermore, this test is a useful tool in detecting the disease since unlike culture and PCR test, it can be performed later in the course of illness (>2 weeks after onset of cough) [2,3]. The objective of this presentation was to conduct the performance verification (accuracy, precision, reproducibility) of the CDC IgG Anti-Pertussis ELISA for use a state public health laboratory setting.

MATERIALS AND METHODS

Reagents: PMP-1 (pertussis master control pool) and pertussis negative control (obtained from the CDC); standards and kit controls (combination of PMP-1, negative control, glycerol); coating buffer, wash buffer; standard and kit control diluent; Peroxidase-labeled mouse monoclonal anti-human IgG conjugate (Hybridoma Reagent Lab, Baltimore, MD); 10% Tween-20; 10X PBS; standard and kit control preparation buffer; TMB Substrate (KPL, Gaithersburg, MD), 1 N HCL; pertussis toxin (Protein Express Inc., Cincinnati, OH). A verification panel (n=15) with known concentration values ranging from <15 IU/ml to >480 IU/ml were obtained from the CDC.

ELISA: The CDC IgG Anti-pertussis Toxin ELISA test was performed following the CDC protocol [2]. High binding, polystyrene, flat-bottom plates were coated with 2 ug/ml of pertussis toxin. The plates were incubated for 24 hours at 4°C, followed by a wash. Standards and kit controls of known concentrations, as well as the verification panel were added to the plates in duplicate. After 2 hours of incubation at 22°C, the conjugate was added followed by another incubation for 2 hours at 22°C. The TMB substrate was added, and the plates were incubated for 15 minutes at 22°C. The reaction was stopped by adding 1N HCL. The plates were read at an Optical Density (OD) of 450nm using the Biotek ELx800 Plate Reader (Biotek Instruments, Highland Park, VT).

Reportable/Cutoff Values: The average OD of each sample was plotted against the concentration using automatic data processing software with a four-parameter logistic (4PL) function (Gen5 2.01, Biotek Instruments, Highland Park, VT). The concentration of each sample was obtained directly from the standard curve. The final concentration (IU/ml) was calculated based on the average of two valid values for each sample. The percent coefficient of variance (%CV) of <25% was considered acceptable. The assay interpretations were: ≥ 94IU/ml (Positive), <49 IU/ml (Negative), and 49-93 IU/ml (Equivocal). The mean, standard deviation and %CV were calculated using Microsoft Excel (Redmond, WA).

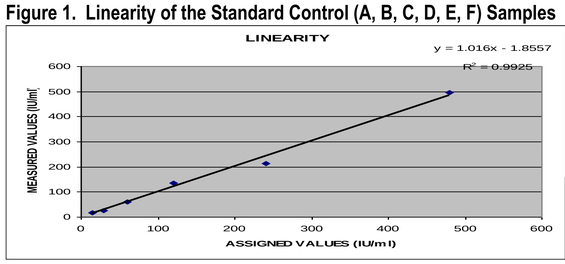
RESULTS

- The CDC IgG Anti-pertussis Toxin ELISA was highly specific with a low variation, hence the assay results were close to the reference values provided by CDC.
- Parallel Verification: The standard and kit controls were prepared at DHMH. The DHMH values were within the acceptable reference (CDC) range (see Table 1). As shown in Figure 1, the geometric mean of the reference (CDC) values in Table 1 were plotted against the DHMH values. A linear correlation was observed.
- Performance Verification:
  - Accuracy: As shown in Table 2, the DHMH values were within the reference (CDC) acceptable range.
  - Precision: The %CV were all acceptable (< 25%; see Table 3). The control samples used were: Positive (Standard F); Equivocal (Standard C); and Negative (C-I).
  - Reproducibility: The results were reproduced for a total of eight days. The %CV were within the acceptable values of < 25% (see Table 4).

Table 1. Parallel Verification of the Standards and Kit Controls

CONTROL SAMPLES	REFERENCE (IU/ml)	DHMH (IU/ml)
C-I	< 15	3
C-II	26-68	31
C-III	56-134	102
Prof	335	317
Prof 1:2	168	181
Prof 1:4	84	71
Prof 1:8	42	2
Standard A	15	16
Standard B	30	27
Standard C	60	60
Standard D	120	137
Standard E	240	213
Standard F	480	496

Figure 1. Linearity of the Standard Control (A, B, C, D, E, F) Samples



expected	measured
15	16
30	27
60	60
120	137
240	213
480	496

Table 2. Accuracy Performance Verification

VERIFICATION PANEL	REFERENCE (IU/ml)	REFERENCE INTERPRETATION	DHMH (IU/ml)	DHMH INTERPRETATION	ACCURACY
S1	≥ 94	Positive	≥ 94	Positive	100%
S2	56	Equivocal	49	Equivocal	100%
S3	≥ 94	Positive	≥ 94	Positive	100%
S4	<49	Negative	<49	Negative	100%
S5	<49	Negative	<49	Negative	100%
S6	92	Equivocal	52	Equivocal	100%
S7	≥ 94	Positive	≥ 94	Positive	100%
S8	<49	Negative	<49	Negative	100%
S9	80	Equivocal	55	Equivocal	100%
S10	≥ 94	Positive	≥ 94	Positive	100%
S11	≥ 94	Positive	≥ 94	Positive	100%
S12	<49	Negative	<49	Negative	100%
S13	<49	Negative	<49	Negative	100%
S14	59	Equivocal	32	Negative*	100%
S15	57	Equivocal	51	Equivocal	100%

*Acceptable result, within 2 standard deviations of the reference (26-92 IU/ml).

Table 3. Precision Performance Verification

	MEAN (OD 450 nm)			STANDARD DEVIATION (OD 450 nm)			% COEFFICIENT OF VARIANCE		
DAY	POS	EQUIVOCAL	NEG	POS	EQUIVOCAL	NEG	POS	EQUIVOCAL	NEG
1	1.520	0.571	0.174	0.134	0.016	0.013	8.789	2.851	7.744
2	1.577	0.763	0.246	0.087	0.035	0.031	5.495	4.543	12.593
3	1.412	0.808	0.205	0.048	0.047	0.030	3.405	5.867	14.487
4	1.667	0.697	0.217	0.081	0.084	0.006	4.880	12.081	2.607
5	1.526	0.869	0.179	0.028	0.094	0.022	1.853	10.828	12.280
6	1.388	0.660	0.165	0.002	0.033	0.008	0.153	5.039	4.728
7	1.364	0.803	0.265	0.018	0.035	0.002	1.348	4.318	0.802
8	1.511	0.922	0.204	0.042	0.035	0.018	2.808	3.760	9.012

Table 4. Reproducibility Performance Verification

CONTROL SAMPLES	MEAN (OD 450 nm)	STANDARD DEVIATION (OD 450 nm)	% COEFFICIENT OF VARIANCE
POS	1.520	0.113	7.457
EQUIVOCAL	0.730	0.108	14.721
NEG	0.201	0.036	17.836

CONCLUSION

Implementation of the CDC IgG Anti-Pertussis Toxin ELISA in a state public health laboratory setting can be a useful tool for surveillance during a pertussis outbreak.

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ACKNOWLEDGEMENTS, DISCLAIMER, CONTACT INFORMATION

I would like to thank all the Vaccine Preventable Diseases Laboratory Scientists (Yvette Washington, Gira Thakore, Tierra Guy) for their assistance, and the Johns Hopkins University Zanvyl Krieger School of Arts and Sciences. Also, I would like to thank Dr. Leena Trivedi, Supervisor of the Vaccine Preventable Diseases Laboratory, as well as the MD DHMH Laboratories Administration Chief of the Division of Virology and Immunology and my mentor, Dr. Maria Paz Carlos, for the opportunity and support of my professional development. The contents of this project are solely the responsibility of the authors and do not necessarily represent the official views of MD DHMH Laboratories Administration, Johns Hopkins University or the CDC.

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